ELSEVIER

Nutrition 22 (2006) 295–302

NUTRITION

www.elsevier.com/locate/nut

Basic nutritional investigation

Effects of Concord grape juice on cognitive and motor deficits in aging

Barbara Shukitt-Hale, Ph.D.[a,*], Amanda Carey, B.S.[a], Laura Simon, B.A.[a], David A. Mark, Ph.D.[b], and James A. Joseph, Ph.D.[a]

[a] *USDA-ARS, Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts, USA*
[b] *Dmarknutrition, Maynard, Massachusetts, USA*

Manuscript received January 20, 2005; accepted July 23, 2005.

Abstract

Objective: Animals and humans show increased motor and cognitive declines with aging that are thought to be due to increased susceptibility to the long-term effects of oxidative stress and inflammation. Previous findings have suggested that reversals in these age-related declines might be accomplished by increasing the dietary intake of polyphenolics found in fruits and vegetables, especially those identified as being high in antioxidant and anti-inflammatory activities.

Methods: We investigated the beneficial effects of two concentrations of Concord grape juice (10% and 50%) compared with a calorically matched placebo for their effectiveness in reversing age-related deficits in behavioral and neuronal functions in aged Fischer 344 rats.

Results: Rats that drank the 10% grape juice from age 19 to 21 mo had improvements in oxotremorine enhancement of K^+-evoked release of dopamine from striatal slices and in cognitive performance on the Morris water maze, and the 50% grape juice produced improvements in motor function.

Conclusions: These findings suggest that, in addition to their known beneficial effects on cancer and heart disease, polyphenolics in foods may be beneficial in reversing the course of neuronal and behavioral aging, possibly through a multiplicity of direct and indirect effects that can affect a variety of neuronal parameters.

Keywords: Polyphenolics; Flavonoids; Antioxidant; Anti-inflammatory; Spatial memory and learning; Phytochemicals; Signaling

Introduction

Oxidative stress (OS) and inflammation are thought to be major factors in brain aging and in age-related neurodegenerative disease [1–4]. Humans and animals show increased motor and cognitive declines with aging [5–7] that are thought to be due to increased susceptibility to the long-term effects of OS and inflammation [4]. Deficits in brain functions due to OS may be due in part to a decline in the endogenous antioxidant defense mechanisms and to the vulnerability of the brain to the deleterious effects of oxidative damage [8,9]. With respect to inflammation, increases in inflammatory mediators (e.g., cytokines) and increased mobilization and infiltration of peripheral inflammatory cells into the brain have been shown to produce deficits in behavior similar to those observed during aging [2]. Further, age-related changes in brain vulnerability to OS and inflammation may be the result of membrane changes and differential receptor sensitivity [10]. Therefore, it would seem that age-related deleterious effects on behavior and brain function could be retarded or even reversed by increasing antioxidant and/or anti-inflammatory levels, and that the synergistic effects of combinations of antioxidants and/or anti-inflammatories, particularly phytochemicals (polyphenolics), might be effective in this regard because polyphenolics possess potent antioxidant properties [11]. Previous findings have suggested that reversals in age-related declines might be accomplished by increasing the dietary intake of fruits and vegetables, especially those identified as being high in antioxidant activity [12,13].

In this regard our laboratory has shown, in several

This study was supported in part by Welch Foods, Inc., Concord, Massachusetts.

* Corresponding author. Tel.: +617-556-3118; fax: +617-556-3222.
E-mail address: barbara.hale@tufts.edu (B. Shukitt-Hale).

0899-9007/06/$ – see front matter
doi:10.1016/j.nut.2005.07.016

studies, that aqueous strawberry, spinach, and particularly blueberry extracts were able to reverse several parameters of brain aging (e.g., deficits in cell communication) such as dopamine (DA) release [14,15] and age-related motor and cognitive deficits when fed to rats from 19 to 21 mo of age [14–17]. These results suggest that polyphenolics in antioxidant-rich foods might be effective in forestalling functional age-related deficits. However, it appears that all fruits and vegetables are not equal in their beneficial properties, and it may be that it is those with very intense color such as berry fruits and grapes that are the most efficacious. Because of the high antioxidant value of Concord grape juice, we felt that it would also be of benefit in reversing or forestalling the deleterious effects of aging on brain function and behavior.

As has been seen with respect to red wine, Concord grape juice is a rich source of flavonoids that include catechins, epicatechins, quercetins, anthocyanins, and proanthocyanidins [18–20] and are potent antioxidants [11,21]. Flavonoids have been shown to have three distinct mechanisms of protection in a cell model: alteration of glutathione metabolism, quenching of reactive oxygen species, and inhibition of calcium influx despite high levels of reactive oxygen species [22]. Further, flavonoids have been shown to be effective in protecting neurons against oxidative insults, possibly by acting selectively within protein and lipid kinase signaling cascades, and not through their potential to act as antioxidants [23,24]. Some flavonoids also have anti-inflammatory properties in that they are potent inhibitors of nitric oxide synthase-2 induction and also increase endothelial nitric oxide synthase-3 activity [25].

In humans, Concord grape juice has been shown to inhibit platelet activity and protect against epinephrine activation of platelets, perhaps by enhancing platelet and endothelial production of nitric oxide [26]. Consumption of Concord grape juice by patients also offered increased protection against low-density lipoprotein cholesterol oxidation, even though many patients were also taking another antioxidant, vitamin E (400 IU/d) [27]. Through these mechanisms, it is thought that the flavonoids in Concord grape juice may inhibit the initiation of atherosclerosis [26–28].

Given these considerations, we investigated the putative beneficial effects of two concentrations of Concord grape juice (10% and 50%) against a calorie-matched placebo product for their effectiveness in reversing age-related deficits in behavioral and neuronal function in 19-mo-old Fischer 344 rats whose sole source of liquid for 9 wk was the experimental products; experimental testing was done at age 21 mo during weeks 6 to 8. There are several reasons we used the 10% strength of juice. First, based on amount per kilogram body weight per day, it is roughly equivalent to the 16 to 20 oz/day of 100% Concord grape juice that has been tested in clinical trials [21,28–30]. Second, it is closer to the amount of oxygen radical absorbance capacity assay [12,13,31] activity we had in the initial diet studies (1.36 mmol of Trolox equivalent per kilogram of diet) [14]. We included the 50% strength to examine if there is a dose-response effect.

Materials and methods

Animals

Forty-five male Fischer 344 rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) were individually housed in stainless steel mesh suspended cages, provided food (NIH-31 diet, product 7017, Harlan Teklad, Madison, IN, USA) and water ad libitum, and maintained on a 12-h light/12-h dark cycle. After a 4-d acclimatization period to the facility, 19-mo-old rats were matched by weight and then randomly assigned to one of three juice groups: 0% grape juice, 10% grape juice, and 50% grape juice (n = 15/group). During the course of the study, one rat in the 0% group and one in the 50% group were removed from the study because of extensive weight loss due to a pituitary tumor and an enlarged spleen.

Grape juice

Rats were given 0%, 10%, or 50% grape juice as their sole source of liquid. The three experimental products were matched for calories and other components by blending 100% Concord grape juice (which contained no added sugar, artificial flavors, or colors) with a placebo formulation matched to grape juice for calories, fructose and sucrose contents, acidity, taste, color, and aroma. The experimental products were provided by Welch Foods (Concord, MA, USA) in glass bottles and kept refrigerated until used. The experimental products were provided to the rats in small glass jars that were secured to their cages to prevent spillage; the juice in the jars was changed daily and the jars were changed twice a week. The 50% juice consisted of 50% Concord grape juice, 0% placebo, and 50% water; the 10% juice consisted of 10% Concord grape juice, 40% placebo, and 50% water; and the 0% juice consisted of 0%

Table 1
Composition of the juice products

	0% Juice	10% Juice	50% Juice
Total phenolics (mg/L gallic acid equivalents)	0	255	1,098
Anthocyanins (mg/L malvidin equivalents)	0	36.6*	183
Proanthocyanidins (mg/L catechin equivalents)	0	37	183
ORAC-FL (μmol/L TE)	207	3,272	16,397

ORAC-FL, oxygen radical absorbance capacity assay; TE, Trolox equivalents

* Calculated value.

Concord grape juice, 50% placebo, and 50% water. All experimental products contained 85 kcal/8 oz (35 kcal/100 mL) and none was a significant source of fat calories, saturated fat, cholesterol, fiber, vitamin C, vitamin A, calcium, or iron.

Table 1 presents additional information on the composition of the experimental products. The concentration of total phenols was determined by the Folin-Ciocalteu procedure [32]. Total phenols were expressed as milligrams per liter of gallic acid equivalents. Anthocyanins were determined by a spectrophotometric method and expressed as milligrams per liter of malvidin equivalents [33]. Proanthocyanidins were determined by normal-phase high-performance liquid chromatography [34] after solid-phase extraction of the juice with a Sephadex LH-20 [35]. Proanthocyanidins were expressed as milligrams per liter of catechin equivalents (R. L. Prior, personal communication). Oxygen radical absorbance capacity was determined by an improved version of the assay using fluorescein as the probe [36]; units were expressed as micromoles per liter of Trolox equivalents.

The 19-mo-old Fischer 344 rats (15/group $\times$ 3 groups, for a total of 45 rats) were given one of these drinks for 6 wk before motor testing and 8 wk before cognitive testing at age 21 mo. Weights were recorded three times during this period and during behavioral testing. Food intake (over a 72-h period) and juice intakes (four times) were also measured during the course of the study. All rats were observed daily for clinical signs of disease. These animals were used in compliance with all applicable laws and regulations and with principles expressed in the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health, U.S. Public Health Service). This study was approved by the animal care and use committee of our center.

Behavioral tests

Psychomotor testing.

A battery of age-sensitive tests of psychomotor behavior [6,7,37] was administered in a randomized order to the animals at the end of the sixth week after grape juice treatment. Each test was performed once, separated by a break between tasks. Rats were tested in a random manner, with the restriction that one rat from each juice group be tested in succession. Briefly, the tests were: 1) rod walking, which measures psychomotor coordination and the integrity of the vestibular system by requiring the animal to balance on a stationary horizontal rod; 2) wire suspension, which measures muscle strength and prehensile reflex, which indicates an animal's ability to grasp a horizontal wire with its forepaws and to remain suspended; 3) plank walking, which measures balance and coordination by exposing the rats to three different sizes of horizontal planks; 4) inclined screen, which measures muscle tone, strength, stamina, and balance by placing the animal on a wire mesh screen that is tilted 60° to the horizontal plane of the floor; and 5) accelerating Rotarod, which measures fine motor coordination, balance, and resistance to fatigue by assessing the duration that a rat can remain standing/walking on a rotating, slowly accelerating rod. For a more detailed description of the tests, see Shukitt-Hale et al.[7].

Cognitive testing.

The Morris water maze is an age-sensitive [5–7] learning paradigm that requires the rat to use spatial learning to find a hidden platform (10 cm in diameter) submerged 2 cm below the surface of the water in a circular pool of water (134 cm in diameter $\times$ 50 cm in height, maintained at 23°C) and to remember its location from the previous trial. Accurate navigation is rewarded with escape from the water onto the platform, that the rat uses distal cues to effectively locate itself. The working memory version of the Morris water maze [5,38] was performed daily for 4 consecutive days during the eighth week of grape juice treatment, with a morning and an afternoon session, two trials each session, with a 10-min intertrial interval between trials. Rats were tested in a random manner, with the restriction that one rat from each group be tested in succession. At the beginning of each trial, the rat was gently immersed in the water at one of four randomized start locations. Each rat was allowed 120 s to escape onto the platform; if the rat failed to escape within this time, it was guided to the platform. Once the rat reached the platform, it remained there for 15 s (trial 1; reference memory or acquisition trial). The rat was returned to its home cage between trials (10 min). Trial 2 (working memory or retrieval trial) used the same platform location and start position as trial 1. Performances were videotaped and analyzed with image tracking software (HVS Image, Twickenham, Middlesex, UK), which allows measurements of latency to find the platform (seconds), path length (centimeters), and swimming speed (centimeters per second; latency/path length). For a more detailed description of the maze and the paradigm used, see Shukitt-Hale et al. [7].

Dopamine release

We previously showed in numerous experiments that the muscarinic enhancement of K^+-evoked DA release (K^+-ERDA) from superfused striatal slices is an indicator of receptor sensitivity and is sensitive to aging and OS [39–42]. The protective capacity of the striatal tissue obtained from the control and supplemented groups was assessed by examining differences in the oxotremorine enhancement of striatal K^+-ERDA. To assess DA release, crosscut (300 μm, McIlwain tissue chopper) striatal slices were obtained from different groups during the ninth week of grape juice treatment. Slices were placed in small glass vials containing modified Krebs-Ringer basal release medium that had been bubbled for 30 min with 95% O_2/5% CO_2 and that contained 21 mM $NaHCO_3$, 3.4 mM glucose, 1.3 mM NaH_2PO_4, 1 mM ethylene glycol tetraacetic acid (EGTA), 0.93 mM $MgCl_2$, 127 mM NaCl, and 2.5 mM KCl (low KCl; pH 7.4). Slices were then placed in the perfusion chambers, where they were maintained at 37°C and perfused with the basal release medium for 30 min. After this equilibration period, the medium was switched to one containing 30 mM KCl, 1.26 mM $CaCl_2 \cdot 2H_2O$ (in place of EGTA), 57 mM NaCl, and 0 or 500 μM oxotremorine and then the enhancement of K^+-ERDA was assessed. DA release was quantitated by high-performance liquid chromatography coupled to electrochemical detection and expressed as picomoles per milligram of protein as determined by the method of Lowry et al. [43].

Statistical analyses

For each behavioral measure, between-subjects analysis of variance models comparing the three juice groups were performed using Systat (SPSS, Inc., Chicago, IL) to test for statistical significance at the $p < 0.05$ level. Days or trials, when appropriate, were included in the model as a within-subjects variable. Post-hoc comparisons, to determine differences among juice groups, were performed using Fisher's least significant difference test post-hoc analysis.

Results

There were no differences in weight across groups at any time during the study and no differences in food or juice intakes across groups over the course of the study (Table 2).

The rats that consumed the 10% and 50% grape juices performed significantly better on the behavioral tests than did control rats; however, which dose of juice was more effective depended on the behavioral endpoint. Specifically, the 50% group showed improved performance on tests of psychomotor performance compared with control (0% juice) rats. On the rod walk (Fig. 1A), latency to fall was significantly higher in the 50% group than in the control group ($P < 0.05$) but not in the 10% group, which was not different from the 50% group ($P = 0.32$) or the control group ($P = 0.24$). In the wire suspension (Fig. 1B) and small plank (Fig. 1C) tests, latency to fall was significantly better in the 50% group than in the control group ($P \leq 0.05$) and the 10% group ($P \leq 0.05$). No group differences were seen on the medium and large plank, inclined screen, or Rotarod tests. The 10% group showed no improvement

Table 2
Body weight at the start and finish of the study and juice and food intakes over the 8-wk study*

	Body weight (g)		Juice intake (mL/d)	Food intake (g/d)
	Start of study	End of study		
0% Juice	435 ± 8	435 ± 9	26.6 ± 1.3	20.2 ± 0.5
10% Juice	438 ± 8	439 ± 8	25.7 ± 0.9	21.7 ± 0.9
50% Juice	434 ± 7	428 ± 10	27.9 ± 1.7	21.6 ± 1.3

* Mean ± standard error of the mean.

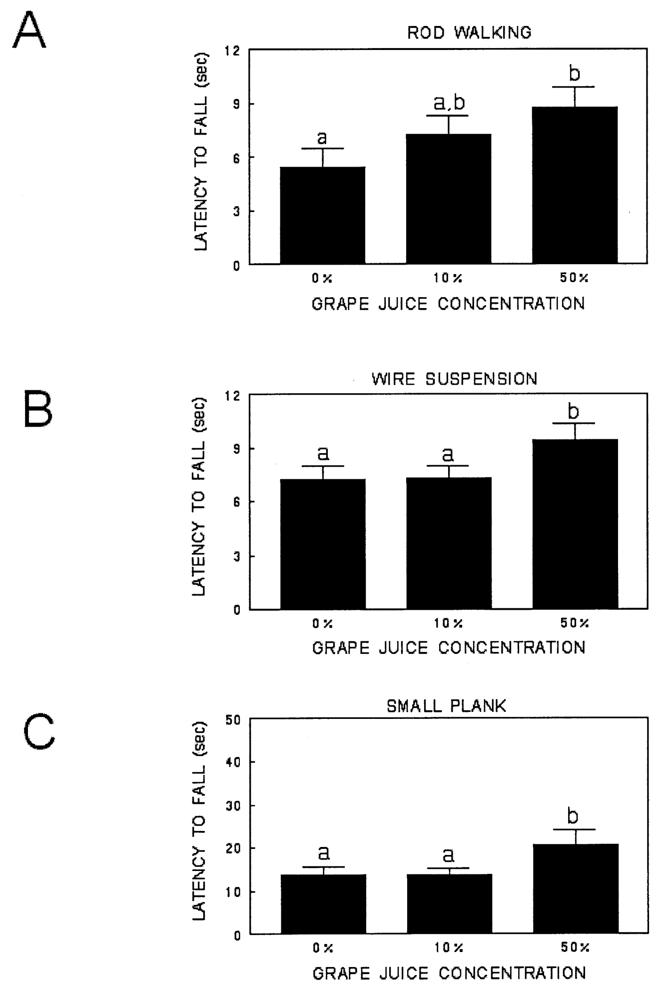

Fig. 1. Latency to fall (mean ± standard error of the mean: seconds) in the rod walking (A), wire suspension (B), and small plank (C) tests for the 0%, 10%, and 50% grape juice groups. Means not sharing a common letter are significantly different from each other ($P < 0.05$, Fisher's least significant difference test).

when compared with the control group on any motor task. Therefore, 50% grape juice supplementation improved performance on motor tests, which rely on balance, coordination, and strength.

When examining cognitive performance, the 10% group showed improved performance over the other groups. We performed separate *t* tests between the two trial latencies and distances for each group for all days to determine whether the different groups significantly improved their performance from trial 1 to trial 2, which would demonstrate improved working memory. The 10% group showed significant ($P < 0.05$) differences in latencies (Fig. 2A) and distances (Fig. 2B) between trials 1 and 2, i.e., trial 2 latencies and distances swum were significantly less than in trial 1, showing that these rats demonstrated one-trial learning, even with the 10-min retention interval. This one-trial learning was not found in the control group or the 50% group. This difference was not due to swim speed because there were no differences between groups on this parameter.

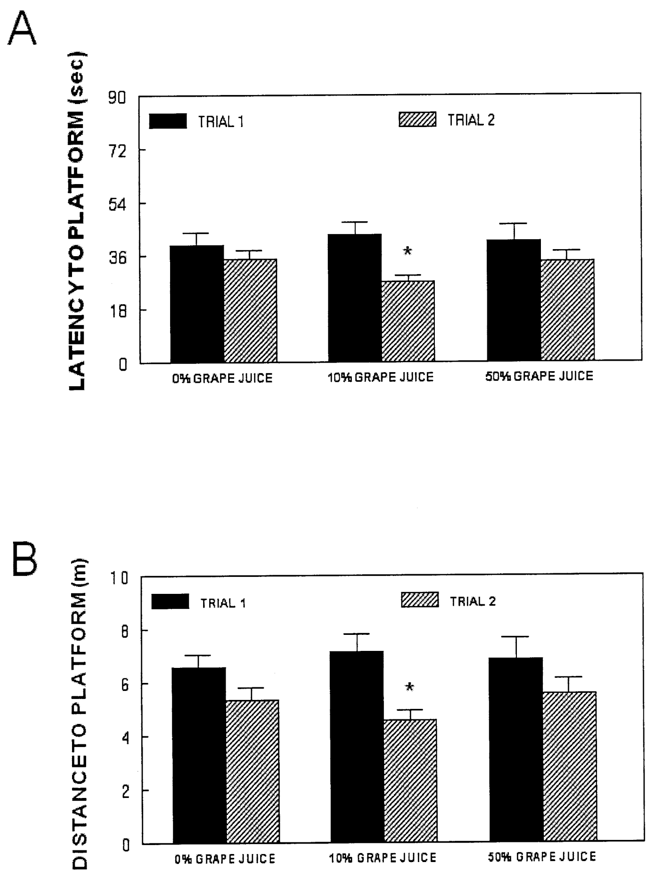

Fig. 2. Morris water maze performance (mean ± standard error of the mean) assessed as latency (A) or distance (B) to find the hidden platform over 4 d of testing. Asterisk indicates a difference in performance (i.e., improvement) from trial 1 to trial 2 for the 10% grape juice group ($P < 0.05$), meaning that the 10% grape juice group had improved working memory. This improvement was not seen in the control rats or the 50% grape juice group.

DA release (oxotremorine-enhanced striatal K^+-ERDA) was significantly greater ($P < 0.05$) in the 10% group than in the control or 50% group (Fig. 3). Therefore, 10% grape juice was more effective in increasing the sensitivity of the muscarinic receptor in the aging rat.

Discussion

It appears from these findings that the effects of Concord grape juice depend on the particular variable assessed and the concentration of the juice. For example, the greatest effects of the grape juice on cognitive performance and striatal DA release were seen at the 10% concentration, whereas the animals maintained on the 50% concentration were no different than controls with respect to these parameters. Age-related alterations in muscarinic control of striatal DA release have been related to spatial memory and motor function [39,41]. However, when motor behavior was assessed, it was clear that the 50% concentration was the most beneficial in restoring performance (e.g., small plank test). These findings suggest that it may take a higher concentration of grape juice to enhance motor performance, whereas lower concentrations may be sufficient to alter cognitive performance. Interestingly, the DA release data were similar to the cognitive data, in that the 10% juice showed improvements, whereas the 50% group did not; however, the 50% juice enhanced behavior in the motor tests. We do not have an explanation as to why the 50% juice did not show positive effects in DA release or cognitive behavior. The effects of the grape juice may be task specific and on some parameters may reflect a U-shaped function.

Our previous research showed that, although other fruits and vegetables (e.g., spinach, strawberries) were effective in altering cognitive performance, only a few (e.g., blueberries, cranberries, boysenberries) enhanced motor performance [14,44,45], suggesting that it may be more difficult to reverse motor deficits than age-induced deficits in cognitive function. The reason for this will require further elaboration and specification of the mechanisms involved in these effects. However, enhancement of motor behaviors may require recruitment of additional signaling pathways and may involve peripheral mediation. For example, we have preliminary data suggesting that blueberry supplementation in senescent animals, in addition to enhancing DA release and several motor behavioral parameters, decreases inflammatory and OS markers in muscle tissue taken from the gastrocnemius and quadriceps of blueberry-supplemented rats (A. Reznick, personal communication). Moreover, Concord grape juice can affect the peripheral vasculature [21,26–28,46]. Such peripheral effects may require larger amounts of the polyphenols contained in fruits such as purple grapes and blueberries. However, skeletal muscle alterations in conjunction with those on the vasculature may also be

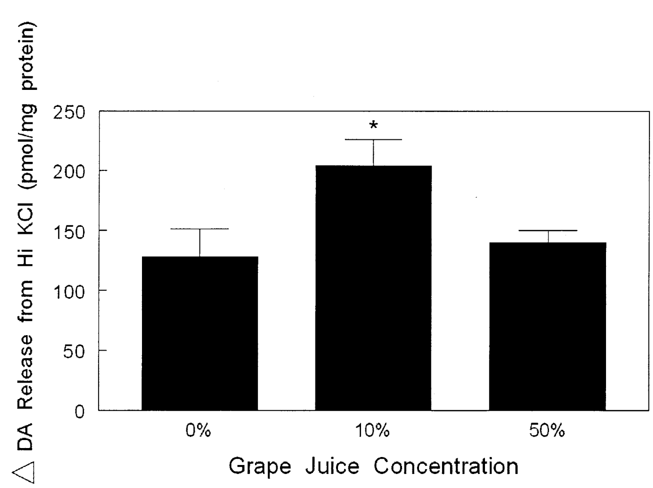

Fig. 3. Oxotremorine enhancement of DA release from striatal slices obtained and prepared from animals maintained on 0%, 10% or 50% juice (mean ± standard error of the mean). The 10% grape juice group is significantly (*$P < 0.05$) better than control and 50% juice groups. ΔDA, change in dopamine.

contributing to the effects on motor performance observed in the present study and should be further explored.

In addition to antioxidant and anti-inflammatory effects [47,48], flavonoids may have a direct effect on cell signaling [49–52]. A study by our group showed that blueberry supplementation protected mice that were transgenic for amyloid precursor protein and presenilin-1 mutations from developing memory deficits (as seen in the Y maze) without altering pathology, i.e., Aβ loads in the hippocampus and frontal cortex [53]. However, preservation of behavioral performance in the blueberry-supplemented transgenic animals involved enhancement of several neuronal signals that are important in learning and memory, such as muscarinic receptor guanosine triphosphatase activity, hippocampal protein kinase-α, and extracellular signal regulated kinase (ERK), because some of these indices were also correlated with Y-maze performance. Therefore, blueberry supplementation may facilitate behavioral performance in this mouse model by enhancing neuronal signaling parameters involved in learning and memory. Multiple studies have shown that protein kinase activity is important in memory formation, in particular spatial memory [54], and that ERK is involved in striatal-dependent learning and memory [55] and hippocampal-dependent spatial memory [56].

Another study by our group showed that changes in hippocampal plasticity parameters such as hippocampal neurogenesis, extracellular receptor kinase activation, insulin-like growth factor-1 (IGF-1), and IGF-1 receptor levels were increased in blueberry-supplemented aged animals [57]. Further, aspects such as proliferation, extracellular receptor kinase activation, IGF-1, and IGF-1 receptor correlated with improvements in spatial memory as measured in a radial arm maze [57]. Therefore, cognitive improvements afforded by polyphenolic-rich fruits such as blueberries appear in part to be mediated by their effects on hippocampal plasticity. The 10% purple grape juice used in the present study may also enhance hippocampal plasticity and through this mechanism improve Morris water maze performance. However, more studies will have to be done to confirm this hypothesis.

There is evidence to suggest that specific grape juice polyphenolics may have direct effects on cell signaling. For example, resveratrol activated ERK-1 and ERK-2 in a neuronal cell model [58]. Other studies have shown that resveratrol inhibits apoptotic cell death by downregulating the nuclear factor-κB and activator protein-1 pathways and the mitochondrial cell death pathway [48]. However, it should be noted that pure resveratrol was used in these previous studies, rather than the grape juice used in the present study, which has smaller quantities of resveratrol, so more work is needed to assess the effects of the Concord grape juice used in this study on cell signaling.

However, even though fruits and vegetables, including the grape juice used in this study, are not pure compounds, this characteristic may be the one that contributes to their diverse and varied mechanisms of action. Therefore, the entire grape, which is a mixture of many different polyphenols, may be more effective than any one single component because individual polyphenols might exert their effects through different and/or independent mechanisms [48]. For example, Conte et al. [47] concluded that plant polyphenols have specific pharmacologic activities that interact with cell-signaling cascades, influence the cell at a transcriptional level, and downregulate pathways that lead to cell death, rather than general properties to scavenge reactive oxygen species and free radicals [47]. Therefore, use of different or complex compounds with synergistic activity may protect more effectively, especially against complex mechanisms of toxicity [47]. It is becoming clear that flavonoids may have a multiplicity of direct and indirect effects that can profoundly affect different neuronal parameters that lead to alterations in motor and cognitive behaviors [59]. In support of the concept of multiple and complementary flavonoid effects beyond simple antioxidant function, a prospective study of dementia in subjects older than 65 years reported an inverse relation between baseline intake of dietary flavonoids and development of dementia [60], whereas a review cited multiple studies reporting no effect from antioxidant nutrients such as vitamin C, vitamin E, and β-carotene when provided in supplement form [61]. It may be that the whole is greater than the sum of its parts.

References

[1] Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, Breteler MM. Dietary intake of antioxidants and risk of Alzheimer disease. JAMA 2002;287:3223–9.

[2] Hauss-Wegrzyniak B, Vannucchi MG, Wenk GL. Behavioral and ultrastructural changes induced by chronic neuroinflammation in young rats. Brain Res 2000;859:157–66.

[3] Hauss-Wegrzyniak B, Vraniak P, Wenk GL. The effects of a novel NSAID on chronic neuroinflammation are age dependent. Neurobiol Aging 1999;20:305–13.

[4] Shukitt-Hale B. The effects of aging and oxidative stress on psychomotor and cognitive behavior. Age Ageing 1999;22:9–17.

[5] Brandeis R, Brandys Y, Yehuda S. The use of the Morris water maze in the study of memory and learning. Int J Neurosci 1989;48:29–69.

[6] Ingram DK, Jucker M, Spangler E. Behavioral manifestations of aging. In: Mohr U, Cungworth DL, Capen CC, editors. Pathobiology of the aging rat. Volume 2. Washington, DC: ILSI Press; 1994, p. 149–70.

[7] Shukitt-Hale B, Mouzakis G, Joseph JA. Psychomotor and spatial memory performance in aging male Fischer 344 rats. Exp Gerontol 1998;33:615–24.

[8] Olanow CW. A radical hypothesis for neurodegeneration. Trends Neurosci 1993;16:439–44.

[9] Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994;74:139–62.

[10] Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: the beneficial properties of fruit polyphenolics. Amer J Clin Nutr 2005; 81(suppl):313S–6S.

[11] Rice-Evans CA, Miller MJ, Bolwell PG, Branley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolics flavonoids. Free Radic Res 1995;22:375–83.
[12] Cao G, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables. J Agric Food Chem 1996;44:3426–31.
[13] Wang H, Cao G, Prior R. Total antioxidant capacity of fruits. J Agric Food Chem 1996;44:701–5.
[14] Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, et al. Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with diets supplemented with blueberry, spinach or strawberry dietary supplementation. J Neurosci 1999;19:8114–21.
[15] Youdim KA, Shukitt-Hale B, Martin A, Wang H, Denisova N, Bickford PC, et al. Short-term dietary supplementation of blueberry polyphenolics: beneficial effects on aging brain performance and peripheral tissue function. Nutr Neurosci 2000;3:383–97.
[16] Bickford PC, Gould T, Briederick L, Chadman K, Pollock A, Young D, et al. Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. Brain Res 2000;866:211–7.
[17] Bickford PC, Shukitt-Hale B, Joseph J. Effects of aging on cerebellar noradrenergic function and motor learning: nutritional interventions. Mech Ageing Dev 1999;111:141–54.
[18] Mattivi F, Zulian C, Nicolini G, Valenti L. Wine, biodiversity, technology, and antioxidants. Ann NY Acad Sci 2002;957:37–56.
[19] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996;20:933–56.
[20] Singletary KW, Stansbury MJ, Giusti M, van Breemen RB, Wallig M, Rimando A. Inhibition of rat mammary tumorigenesis by Concord grape juice constituents. J Agric Food Chem 2003;51:7280–86.
[21] O'Byrne DJ, Devaraj S, Grundy SM, Jialal I. Comparison of the antioxidant effects of Concord grape juice flavonoids and α-tocopherol on markers of oxidative stress in healthy adults. Am J Clin Nutr 2002;76:1367–74.
[22] Ishige K, Schubert D, Sagara Y. Flavonoids protect neuronal cells by three different mechanisms. Free Radic Biol Med 2001;30:433–46.
[23] Schroeter H, Spencer JPE, Rice-Evans C, Williams RJ. Flavonoids protect neurons from oxidative low-density-lipoprotein–induced apoptosis involving c-Jun N-terminal kinase (JNK), c-Jun and caspase-3. Biochem J 2001;358:547–57.
[24] Williams RJ, Spencer JPE, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med 2004;36:838–49.
[25] Olszanecki R, Gebska A, Kozlovski VI, Gryglewski RJ. Flavonoids and nitric oxide synthase. J Physiol Pharmacol 2002;53:571–84.
[26] Folts JD. Potential health benefits from the flavonoids in grape products on vascular disease. Adv Exp Med Biol 2002;505:95–111.
[27] Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation 1999;100:1050–5.
[28] Chou EJ, Keevil JG, Aeschlimann S, Wiebe DA, Folts JD, Stein JH. Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. Am J Cardiol 2001;88:553–5.
[29] Albers AR, Varghese S, Vitseva O, Vita JA, Freedman JE. The anti-inflammatory effects of purple grape juice consumption in subjects with stable coronary artery disease. Atheroscler Thromb Vasc Biol 2004;24:179–80.
[30] Freedman JE, Parker C III, Li L, Perlman JA, Frei B, Ivanov V, et al. Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation 2001;103:2792–8.
[31] Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, et al. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of Vaccinium species. J Agric Food Chem 1998;46:2586–93.
[32] Singleton VL, Rossi JL. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144–58.
[33] Niketic-Aleksic GK, Hrazdina G. Quantitative analysis of the anthocyanin content in grape juices and wines. Lebensm-Wiss U Technol 1972;5:163–5.
[34] Adamson GE, Lazarus SA, Mitchell AE, Prior RL, Cao G, Jacobs PH, et al. HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. J Agric Food Chem 1999;47:4184–8.
[35] Gu L, Kelm M, Hammerstone JF, Beecher G, Cunningham D, Vannozzi S, Prior RL. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. J Agric Food Chem 2002;50:4852–60.
[36] Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J Agric Food Chem 2001;49:4619–26.
[37] Joseph JA, Bartus RT, Clody DE, Morgan D, Finch C, Beer B, et al. Psychomotor performance in the senescent rodent: Reduction of deficits via striatal dopamine receptor up-regulation. Neurobiol Aging 1983;4:313–9.
[38] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11:47–60.
[39] Joseph JA, Dalton TK, Hunt WA. Age-related decrements in the muscarinic enhancement of K^+-evoked release of endogenous striatal dopamine: An indicator of altered cholinergic-dopaminergic reciprocal inhibitory control in senescence. Brain Res 1988;454:140–8.
[40] Joseph JA, Dalton TK, Roth GS, Hunt WA. Alterations in muscarinic control of striatal dopamine autoreceptors in senescence: a deficit at the ligand-muscarinic receptor interface? Brain Res 1988;454:149–55.
[41] Joseph JA, Kowatch MA, Maki T, Roth GS. Selective cross activation /inhibition of second messenger systems and the reduction of age-related deficits in the muscarinic control of dopamine release from perifused rat striata. Brain Res 1990;537:40–8.
[42] Joseph JA, Villalobos-Molina R, Denisova N, Erat S, Cutler R, Strain JG. Age differences in sensitivity to H_2O_2- or NO-induced reductions in K^+-evoked dopamine release from superfused striatal slices: reversals by PBN or Trolox. Free Radic Biol Med 1996;20:821–30.
[43] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:256–75.
[44] Galli RL, Shukitt-Hale B, Bielinski DF, Andres-Lacueva C, Joseph JA. Dietary supplementation with fruit polyphenolics ameliorates age-related deficits in behavior and neuronal markers of inflammation and oxidative stress. Soc Neurosci Abstr 2002;28:294.17.
[45] Shukitt-Hale B, Galli RL, Meterko V, Carey A, Bielinski DF, McGuie T, et al. Dietary supplementation with fruit polyphenolics ameliorates age-related deficits in behavior and neuronal markers of inflammation and oxidative stress. Age 2005;27:49–57.
[46] Vinson JA, Teufel K, Wu N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. Atherosclerosis 2001;156:67–72.
[47] Conte A, Pellegrini S, Tagliazucchi D. Synergistic protection of PC12 cells from beta-amyloid toxicity by resveratrol and catechin. Brain Res Bull 2003;62:29–38.
[48] Sun AY, Simonyi A, Sun GY. The "French Paradox" and beyond: neuroprotective effects of polyphenols. Free Radic Biol Med 2002;32:314–8.
[49] Kong AN, Yu R, Chen C, Mandlekar S, Primiano T. Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. Arch Pharm Res 2000;23:1–16.
[50] Moon SK, Cho GO, Jung SY, Gal SW, Kwon TK, Lee YC, et al. Quercetin exerts multiple inhibitory effects on vascular smooth muscle cells: role of ERK 1/2, cell cycle regulation and matrix metalloproteinase-9. Biochem Biophys Res Commun 2003;301:1069–78.

[51] Schroeter H, Boyd C, Spencer JE, Willams R, Cadenas E, Rice-Evans C. MAPK signaling in neurodegeneration: influences of flavonoids and nitric oxide. Neurobiol Aging 2002;23:861–80.
[52] Stahl W, Ale-Agha N, Polidori MC. Non-antioxidant properties of carotenoids. Biol Chem 2002;383:553–8.
[53] Joseph JA, Arendash G, Gordon M, Diamond D, Shukitt-Hale B, Morgan D. Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer's disease model. Nutr Neurosci 2003;6:153–62.
[54] Micheau J, Riedel G. Protein kinases: which one is the memory molecule? Cell Mol Life Sci 1999;55:534–48.
[55] Mazzucchelli C, Brambilla R. Ras-related and MAPK signalling in neuronal plasticity and memory formation. Cell Mol Life Sci 2000; 57:604–11.
[56] Selcher JC, Atkins CM, Trzaskos JM, Paylor R, Sweatt JD. A necessity for MAP kinase activation in mammalian spatial learning. Learn Mem 1999;6:478–90.
[57] Casadesus G, Shukitt-Hale B, Stellwagen HM, Zhu X, Lee H-G, Smith MA, Joseph JA. Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. Nutr Neurosci 2004;7:309–16.
[58] Tredici G, Miloso M, Nicolini G, Galbiati S, Cavalletti G, Bertelli A. Resveratrol, map kinases and neuronal cells: might wine be a neuroprotectant? Drugs Exp Clin Res 1999;25:99–103.
[59] Youdim KA, Shukitt-Hale B, Joseph JA. Flavonoids and the brain: interactions at the blood brain barrier and their physiological effects on the CNS. Free Radic Biol Med 2004;37:1683–93.
[60] Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues JF. Intake of flavonoids and risk of dementia. Eur J Epidemiol 2000;16:357–63.
[61] Laurin D, Masaki KH, Foley DJ, White LR, Launer LJ. Midlife dietary intake of antioxidants and risk of late-life incident dementia: the Honolulu-Asia Aging Study. Am J Epidemiol 2004;159: 959–67.